Enhanced aggressive phenotype of Tph2 knockout rats is associated with diminished 5-HT1A receptor sensitivity

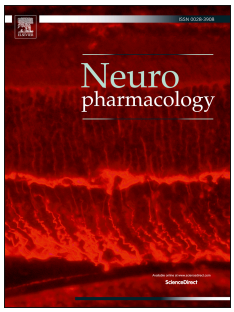
Peeters, D G A; Terneusen, A; de Boer, S F; Newman-Tancredi, A; Varney, M A; Verkes, R J; Homberg, J R

Published in:
Neuropharmacology

DOI:
10.1016/j.neuropharm.2019.05.004

Publication date:
2019

Accepted Manuscript



Enhanced aggressive phenotype of Tph2 knockout rats is associated with diminished 5-HT_{1A} receptor sensitivity

D.G.A. Peeters, A. Terneusen, S.F. de Boer, A. Newman-Tancredi, M.A. Varney, R.J. Verkes, J.R. Homberg

PII: S0028-3908(19)30154-6

DOI: https://doi.org/10.1016/j.neuropharm.2019.05.004

Reference: NP 7627

To appear in: *Neuropharmacology*

Received Date: 29 January 2019

Revised Date: 18 March 2019

Accepted Date: 5 May 2019

Please cite this article as: Peeters, D.G.A., Terneusen, A., de Boer, S.F., Newman-Tancredi, A., Varney, M.A., Verkes, R.J., Homberg, J.R., Enhanced aggressive phenotype of Tph2 knockout rats is associated with diminished 5-HT_{1A} receptor sensitivity, *Neuropharmacology* (2019), doi: https://doi.org/10.1016/j.neuropharm.2019.05.004.

Enhanced aggressive phenotype of Tph2 knockout rats is associated with diminished 5-HT_{1A} receptor sensitivity

D.G.A. Peeters[a,b], A. Terneusen[a], S.F. de Boer[c], A. Newman-Tancredi[d], M.A. Varney[d], R.J. Verkes[b]*, J.R. Homberg[a]*

[a]Dept of Cognitive Neuroscience, Donders Institute for Brain, Cognition and Behaviour, Nijmegen

[b]Dept of Psychiatry, Radboud University Medical Centre, Nijmegen

[c]Dept of Behavioural Neurobiology, Groningen Institute for Evolutionary Life Sciences, Groningen

[d]Neurolixis Inc, Dana Point, CA, USA

*Both authors contributed equally to this work

Corresponding authors:

Judith Homberg; Department of Cognitive Neuroscience, Donders Institute for Brain, Cognition and Behaviour, Radboud University Medical Center, Nijmegen, The Netherlands, 9101, 6500 HB Nijmegen. E-mail address: judith.homberg@radboudumc.nl

Deborah Peeters; Department of Cognitive Neuroscience, Donders Institute for Brain, Cognition and Behaviour, Radboud University Medical Center, Nijmegen, The Netherlands, 9101, 6500 HB Nijmegen. E-mail address: Deborah.Peeters@radboudumc.nl

Abstract

Brain serotonin (5-HT) plays a key role in aggressive behaviours and related psychopathologies, but its precise mechanism of action remains elusive. Genetic animal models may provide a tool to elucidate the relationship between aggression and serotonin. The present study showed that tryptophan hydroxylase 2 (Tph2) knockout (KO) rats, which exhibit profoundly diminished extracellular serotonin levels, display increased aggressiveness compared to their Tph2 wildtype (WT) counterparts. However, the level of aggression in Tph2 KO rats did not equal that of feral wild type Groningen (WTG) rats. To investigate whether enhanced 5-HT_{1A} receptor functionality may be present in Tph2 KO rats, we tested the acute anti-aggressive potency of the highly selective 5-HT_{1A} receptor full agonist NLX-112 (a.k.a. befiradol or F13640). Data show that compared to Tph2 WT and WTG rats, the NLX-112 dose-effect curve was shifted to the right in Tph2 KO animals. These results suggest that, unlike previous reports in Tph2 KO mice, Tph2 KO rats have a decreased 5-HT_{1A} receptor sensitivity compared to both Tph2 WT and WTG animals.

Keywords: resident intruder test, tryptophan hydroxylase 2, 5-HT_{1A} receptor

1. Introduction

There is a clear association between aggressive behaviour and the neurotransmitter serotonin (5-hydroxytryptamine, 5-HT), but the functional complexity of the serotonergic system makes the precise role unclear. Although an inverse relation between basal brain 5-HT levels and aggression is inferred from a vast database of both human (see Duke, Bègue, Bell, & Eisenlohr-Moul, 2013 for meta-analysis) and animal research (Carrillo et al., 2009; Comai et al., 2012; Takahashi et al., 2011), this simple serotonin-deficiency hypothesis of exaggerated aggression is gradually being revised by a conceptual framework that accommodates the increasing knowledge about the complex modulatory and regulatory mechanisms of brain 5-HT activity (De Boer and Newman-Tancredi, 2016; Montoya et al., 2012; Olivier, 2004; Rosell and Siever, 2015).

In line with the inverse relationship between 5-HT and aggression, animals chronically treated with selective serotonin reuptake inhibitors (SSRIs) (Caldwell and Miczek, 2008; Perreault et al., 2003; Pinna et al., 2003; Sánchez and Meier, 1997) as well as genetically modified serotonin transporter (SERT) knockout mice and rats (Holmes et al., 2002; Homberg et al., 2007) show decreased aggression together with enhanced brain 5-HT levels. Accordingly, genetically modified tryptophan hydroxylase 2 (Tph2) knockout (KO) mice that show profoundly decreased amounts of brain 5-HT levels, due to the absence of the central nervous system specific and rate-limiting enzyme Tph2, demonstrate exaggerated aggressive behavior (Mosienko et al., 2012). Despite the absence of brain 5-HT synthesis, serotonergic markers other than Tph2 are preserved in these animals (Gutknecht et al., 2012, 2008; Kriegebaum et al., 2010).

Among the multiple mechanisms that control 5-HT neurotransmission, the 5-HT_{1A} receptor subtype plays a prominent role as shown in both human (Coccaro et al., 1990; Parsey et al., 2002; Witte et al., 2009) and animal research (de Boer and Koolhaas, 2005; Korte et al., 1996; Miczek et al., 1998; Takahashi et al., 2011). This receptor is just as 5-HT itself well conserved among mammalian species (Nichols, David E., 2008) and crucially important in regulating serotonergic neuronal (re)activity and consequent 5-HT transmitter synthesis and release processes via its direct (i.e., short-loop somatodendritic autoreceptor function) and indirect (i.e., long-loop heteroreceptor) inhibitory feed-back action on 5-HT neuronal firing (Celada et al., 2001; Hajo and Sharp, 1999; Pineyro and Blier, 1999). Specifically, inhibitory 5-HT_{1A} autoreceptors have been suggested in animal studies to have an important causal role in aggressive responding and in mediating the robust anti-aggressive actions of 5-HT_{1A} receptor agonists (Cooper et al., 2009; de Boer et al., 2000; de Boer and Koolhaas, 2005; De Boer and Newman-Tancredi, 2016; Miczek et al., 1998).

High trait-like aggressive rats and mice are characterized by enhanced somatodendritic 5-HT_{1A} autoreceptor sensitivity (Caramaschi et al., 2007; Cooper et al., 2009; de Boer and Koolhaas, 2005; De Boer and Newman-Tancredi, 2016; Schiller et al., 2006). Accordingly, the diminished aggressive phenotype of SERT deficient rats and mice (Holmes et al., 2002; Homberg et al., 2007) is associated with a sustained increase in extracellular serotonin levels, which could be enabled by desensitization of 5-HT_{1A} autoreceptors (Homberg et al., 2008; Snoeren, 2010). In addition, transgenic mice with conditional overexpression of somatodendritic 5-HT_{1A} autoreceptors exhibit a hyper-aggressive behavioral phenotype (Audero et al., 2013). Finally, enhanced 5-HT_{1A} autoreceptor functioning as shown by decreased neuron firing after 5-HT_{1A} agonist administration was recently reported in Tph2 KO mice that demonstrate high aggressiveness (Araragi et al., 2013; Mlinar et al., 2017).

In contrast to the association between high aggression, low tonic 5-HT and increased 5-HT_{1A} autoreceptor functioning, 5-HT_{1A} receptor agonists show robust anti-aggressive effects in rodents (de Boer et al., 2000; de Boer and Koolhaas, 2005; Miczek et al., 1998; Sakaue et al., 2002). Although current techniques are unsuited to measure 5-HT levels during an aggressive interaction, it was shown that shortly after an aggressive encounter extracellular 5-HT decreased in the prefrontal cortex (van Erp and Miczek, 2000). Therefore, it is likely that 5-HT levels may increase right before or during this acute state of arousal (Beekman et al., 2005; de Boer et al., 2000). Thus, the acute anti-aggressive effect of systemic 5-HT_{1A} receptor agonists is considered to be largely expressed via their efficacious action on the inhibitory autoreceptors located on the soma and dendrites of raphe 5-HT neurons, by attenuating conflict-activated 5-HT neuronal activity in rodent studies (De Almeida and Lucion, 1997; De Boer and Newman-Tancredi, 2016; Mos et al., 1993). Indeed, direct pharmacological activation of 5-HT_{1A} autoreceptors in the dorsal raphe nucleus employing local microinjection of 5-HT_{1A} agonists that potently suppress 5-HT neuronal activity, consistently reduced aggressive behavior in mice and rats (De Almeida and Lucion, 1997; Mos et al., 1993; van der Vegt et al., 2003). However, also local microinjection of a 5-HT_{1A} agonist in the ventral orbital prefrontal cortex reduced aggression in mice (Centenaro et al., 2008; Stein et al., 2013). So targeting both pre- and postsynaptic 5-HT_{1A} receptors may be a good strategy to decrease aggressive behavior.

In the present study we aimed to further elucidate 5-HT_{1A} receptor functioning as shown by decreased aggressive behavior in relation to basal levels of brain 5-HT availability. We used the recently generated Tph2 KO rat model that is deficient in brain 5-HT synthesis and levels (Kaplan et al., 2016).

Their anticipated elevated aggressive phenotype was compared to the aggression levels of a feral strain of wild-type Groningen (WTG) rats, which demonstrate considerable higher levels of offensive aggression than the commonly-used docile strains of laboratory rats (Koolhaas et al., 2013). To assess

functional activity of 5-HT_{1A} receptors, the anti-aggressive potency of the novel highly selective 5-HT_{1A} receptor agonist NLX-112 was determined and compared among the rat strains. We expected an enhanced 5-HT_{1A} receptor sensitivity in these high aggressive Tph2 KO rats in comparison to both Tph2 WT and WTG rats, due to their increased aggression and absent brain 5-HT availability.

2. Materials and methods

2.1 Animals

2.1.1 Tph2 KO and WT rats

Nine Tph2 knockout (KO) (weight 237 ± 35 g) and nine Tph2 wildtype (WT) (247 ± 12 g) rats of 4-5 months of age were used as residents in the acute dose response experiments. These animals were outcrossed for at least 10 generations with wild type Dark Agouti rats (Envigo, The Netherlands). Eighteen Dark Agouti OlaHsd female rats (167±14 g) (Envigo; Horst, the Netherlands) were used as housing companions together with the residents to avoid social isolation and to facilitate territorial behavior. These pairs stayed the same during the course of all experiments, to reduce stress of an unfamiliar partner. Thirty-six Dark Agouti OlaHsd male rats (205±21 g) (Envigo; Horst, The Netherlands) were used as intruders in the resident-intruder test.

2.1.2 WTG rats

Twenty-four Wild type Groningen (WTG) 4-5 months of age (435 ± 5 g) rats (*Rattus Norvegicus;* originally wild-trapped animals bred under conventionalized conditions for 52 generations) were used as residents in the acute dose response experiments. Oviduct-ligated WTG female rats (327 ± 6 g) were used as housing companions to avoid social isolation and to facilitate territorial behavior. These pairs stayed the same during the course of all experiments, to reduce stress of an unfamiliar partner. Group-housed male WTG rats (426 ± 7 g) were used as intruders in the resident-intruder test.

All animals were housed in temperature (21±1 °C) and humidity-controlled (45-60% relative humidity) rooms, with a 12 h reversed light-dark cycle (lights on at 8:00 PM). Experiments were performed in the dark-phase between 9:00 AM and 5:00 PM. Animals were housed under standard social housing conditions in type IV open cages (48 x 38 x 21 cm), unless stated otherwise. For all cages sawdust bedding and cage enrichment was provided, and the animals had *ad libitum* access to water and rodent chow (V1534, Sniff, long-cut pellet, Bio Services, Uden, The Netherlands).

All experiments were approved by the Committee for Animal Experiments of the Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands, and the Groningen University, Groningen, The Netherlands. All efforts were made to minimize animal suffering and to reduce the number of animals used.

2.2 Drugs

NLX-112 (F13640; befiradol) fumarate was supplied by Neurolixis Inc. (Dana Point, CA, USA). This novel agonist is an interesting alternative to previous 5-HT_{1A} receptor agonists as it shows nanomolar affinity, high selectivity and increased efficacy at 5-HT_{1A} receptors (Colpaert et al., 2002; Newman-Tancredi et al., 2017). NLX-112 was administered 20 min before the start of the resident intruder interaction either via intraperitoneal (i.p.) administration (Tph2 KO and WT experiments) or subcutaneous (s.c.) administration (WTG experiments). In between the drug test-days three washout days were scheduled where the animals were left undisturbed. Every animal received all six treatments (i.e. vehicle, 0.01, 0.04, 0.16, 0.63 and 2.5 mg/kg bodyweight) of NLX-112 in a random order. Six different treatment order groups were created, and the 18 Tph2 and 24 WTG animals were randomly assigned to these groups. This resulted respectively in three and four animals per treatment order group. In this way a block design of six different treatments and six different treatment order groups (including three or four animals per group) was created. Doses are expressed as mg/kg of the drug base.

2.3 Experimental procedures

2.3.1 Resident intruder test

An adapted version of the resident-intruder paradigm was used as described by Koolhaas et al., 2013. One week before the experiment male residents were housed together with companion females in a PhenoTyper 4500 cage (45x45x55 cm; Tph2 KO and WT rats) (Noldus, Wageningen, the Netherlands) or large observation cage (80 x 55 x 50 cm; WTG rats) to allow sufficient space for the full range of aggressive behaviors during the aggressive interactions. Females were sterilized by ligation of the oviducts, to keep them hormonally intact and receptive for the males. Bedding material was not cleaned during the experiment to ensure an undisturbed territory.

After one week of habituation, the baseline level of offensive behavior was tested on 3 consecutive days during maximally a 10 min confrontation with an unfamiliar male conspecific in the home territory of the experimental rat. The female partner of the experimental rat was removed from the observation cage approximately 60 min prior to the start of this social provocation test. During the first 3 tests, the intruder was removed immediately after the first attack from the resident occurred, and the attack latency time (ALT) was noted. When no attack occurred within 10 min the intruder was removed and an attack latency time of 600 s was scored. Experimental groups were balanced on the basis of the ALT and the level of offensive behavior performed during the fourth baseline test (day 4), during which the full range of behaviors was recorded and analyzed (see below). After every

interaction, females and cage accessories were returned and animals were left undisturbed until the next test day.

During the resident-intruder agonistic confrontations on the non-drug test-days and on the drug test-days, the full range of behaviors of the experimental resident was video recorded and manually scored offline using Observer XT12 (Noldus, Wageningen, the Netherlands) by an experimenter blind to treatment conditions. An extensive description of the different behavioral elements displayed during agonistic interactions has been reported previously (De Boer et al., 2003; Koolhaas et al., 2013) Briefly, the following behavioral elements are distinguished: 1) lateral threat; 2) keep down; 3) clinch attack; 4) chase; 5) offensive upright; 6) offensive move towards; 7) mounting; 8) social behavior; 9) submissive behavior; 10) other no contact behaviors. The durations of the different behavioral elements were determined and expressed as a percentage of the total 10 minutes of the confrontation. To promote a clear representation of the data, the elements lateral threat, keep down, clinch attack, chase, offensive upright, offensive move towards and mounting are taken as one behavioral category, i.e., percentage of time showing aggressive behavior. Also the latency time to the first attack by the resident was taken as a measure of aggressive initiative/motivation. The term social behavior describes behavior initiated by the resident and describes social move towards behavior, social upright behavior, ano-genital sniffing and all other direct contact between animals such as sniffing and grooming.

2.4 Statistical analysis

Independent t-tests were applied to compare baseline aggression levels between Tph2 KO and WT animals. To compare effects of different doses on 'aggressive behavior', 'attack latency' and 'social behavior' we used a linear mixed model using NLX-112 dose as the within-subject factor (i.e., vehicle, 0.01, 0.04, 0.16, 0.63 and 2.5 mg/kg), time (the sequential number of test days for each drug dose (i.e. 0-6)) as covariate factor and for the Tph2 study, genotype (i.e. Tph2 KO and WT) as between-subject factor. Post-hoc comparisons between genotypes were performed using an independent samples t-test, and dependent samples t-tests were used to compare between doses within groups. In the dose-response studies, the drug effects on aggressive behavior were also computed as percentage of the respective vehicle-treated control values to enable comparisons between the various animal strains using a linear mixed model with NLX-112 dose (i.e., vehicle, 0.01, 0.04, 0.16, 0.63 and 2.5 mg/kg) as the within-subject factor, group (i.e. WTG, Tph2 KO and WT) as between-subject factor and time (the sequential number of test days for each drug dose (i.e. 0-6)) as covariate factor. Post-hoc comparisons between the three groups were done using Tukey's HSD test. Log-logistic analysis was used to estimate the dose of NLX-112 to decrease aggression by 50% (ED50) when expressed as percentage of the respective vehicle-treated control values. This was done using

the optimal curve fit (Three Parameters Logistic Regression (Finney and Tattersfield, 1952)) in the add-on package drc (version 3.0-1) in RStudio (version 1.0.153, 2009-2017). All other statistical analyses were performed using SPSS version 23 (SPSS Inc., Chicago, IL, USA). All data were checked for outliers and normality (using the Shapiro–Wilkinson test). All statistical tests were performed two-sided. All results are expressed as average values ± SEM (standard error of the mean), unless stated differently. Level of significance was set at $p < 0.05$ and $0.05<p\leq0.08$ was considered as a trend of significance.

3. Results

3.1 Genotype effect baseline aggression

At the start of the experiment, resident-intruder tests were repeated three times to establish a stable aggression level. At the fourth test day a full repertoire of aggressive behaviors was recorded to quantify the baseline aggression level. Tph2 KO animals showed increased time spent on aggressive behavior ($t(9.35)=2.93$, $p=0.016$) and a decreased attack latency ($t(13.63)=-2.55$, $p=0.023$) compared to Tph2 WT animals (see Figure 1).

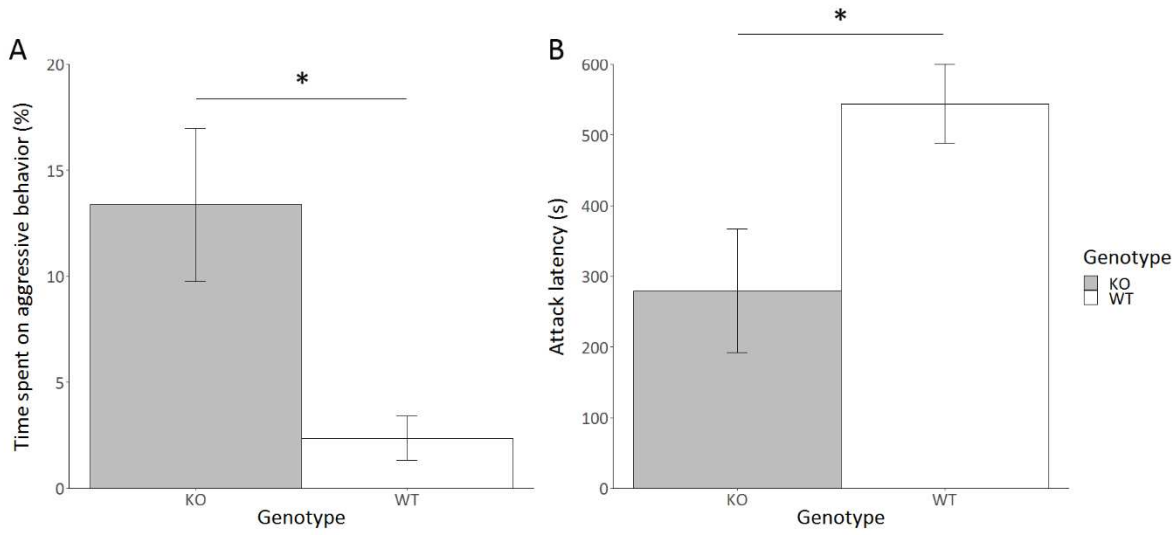


*Figure 1: Baseline aggressive behavior expressed as A) time spent on aggressive behavior (%) and B) attack latency (s) per genotype (Tph2 KO n=9, Tph2 WT n=9) in a ten-minute interaction represented as mean (±SEM). Significant genotype effects indicated by * (p<0.05).*

3.2 Acute anti-aggressive effect NLX-112 in Tph2 KO vs. WT animals

Three days after the last baseline resident-intruder test, animals were subjected to a randomized NLX-112 dose-response treatment regimen to test for effects on 'aggressive behavior', 'attack latency' and 'social behavior'. No interaction or time effects were found (See Figure 2).

There was a significant overall main effect of Tph2 genotype on time spent on aggressive behavior ($F(1, 91)=8.56$, $p=0.004$) and social behavior ($F(1,91)=3.87$, $p=0.052$), whereas this did not reach significance for attack latency ($F(1,91)=3.12$, $p=0.081$). Post-hoc tests revealed an increase in aggression ($t(8)=2.63$, $p=0.030$) and a decrease in social behavior ($t(10,70)=2.27$, $p=0.045$) in KO as compared to WT rats at dose 0.16 mg/kg.

Main effects of NLX-112 doses were found for time spent on aggression ($F(5,91)=2.74$, $p=0.024$), attack latency ($F(5,91)=6.76$, $p<0.001$) and time spent on social behavior ($F(5, 91)=2.69$, $p=0.026$).

Aggressive behavior decreased relative to the basal levels seen under vehicle treatment for doses 0.63 ($t(8)=2.57$, $p=0.033$) and 2.5 ($t(8)=3.48$, $p=0.008$) mg/kg in KO animals. Decreases in aggressive behavior between consecutive doses were found for 0.16-0.63 ($t(8)=2.47$, $p=0.039$) and 0.63-2.5 ($t(8)=2.44$, $p=0.041$) mg/kg in KO animals. The only difference in aggressive behavior between doses in WT animals was found between 0.04-0.16 ($t(8)=3.60$, $p=0.007$) mg/kg.

An increase in attack latency was found only consecutively for 0.01-0.04 mg/kg in KO animals ($t(8)=-2.26$, $p=0.053$), whereas this did not reach significance for WT animals ($t(8)=-2.02$, $p=0.078$).

Social behavior in KO animals increased relative to the basal levels seen under vehicle treatment for doses 0.01 ($t(8)=-3.69$, $p=0.006$), 0.16 ($t(8)=-5.60$, $p<0.001$), and 0.63 ($t(8)=-2.89$, $p=0.020$). Further consecutive dose differences in social behavior were found between doses 0.63-2.5 ($t(8)=3.09$, $p=0.015$) mg/kg, where time spent on social behavior decreased in KO animals. No differences were found for WT animals.

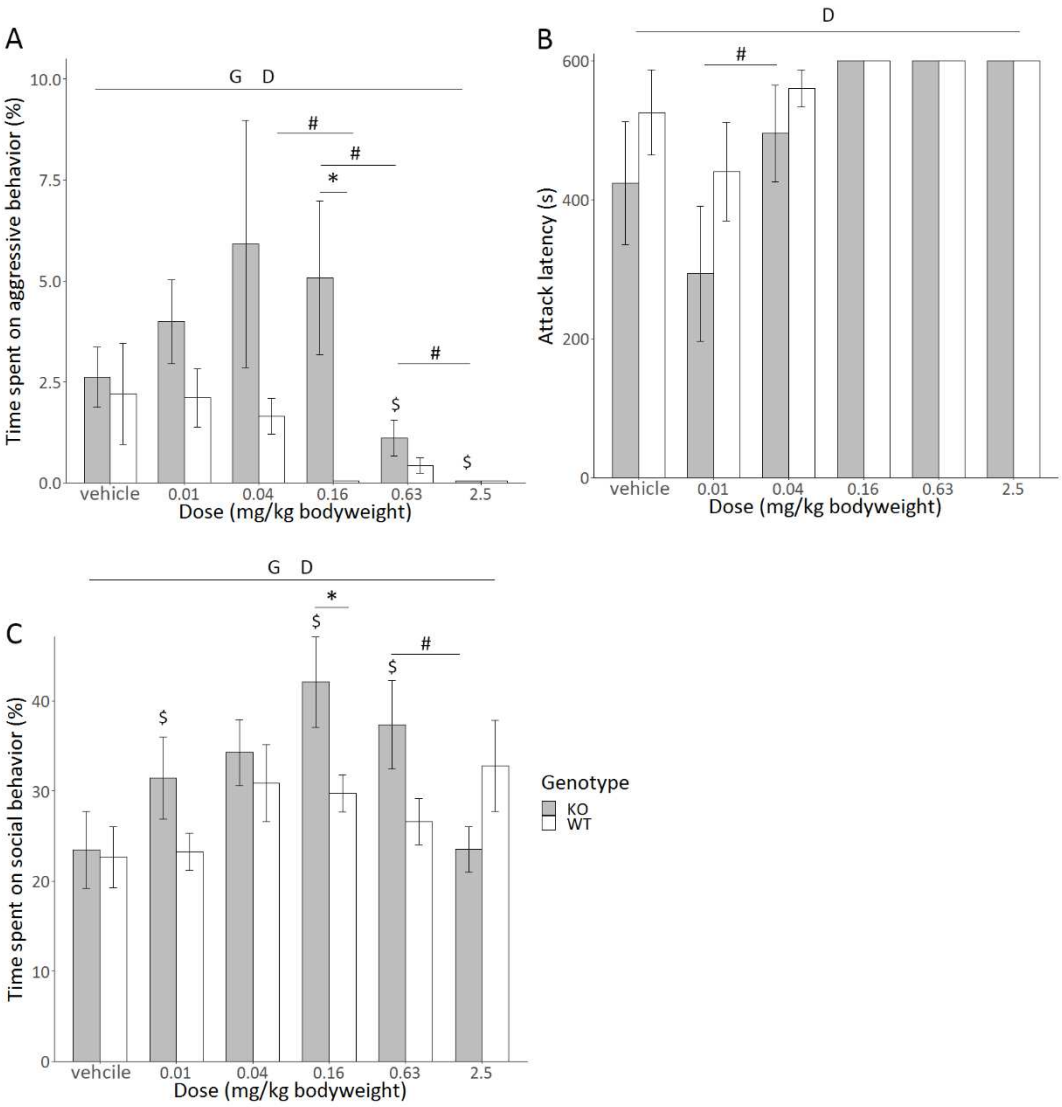


*Figure 2: Effect of different dosages of NLX-112 (vehicle, 0.01, 0.04, 0.16, 0.63, 2.5 mg/kg bodyweight) on A) time spent on aggressive behavior, B) attack latency and C) time spent on social behavior in resident Tph2 KO (n=9) and resident Tph2 WT (n=9) rats during a ten-minute interaction with an intruder animal. Data are represented as mean (±SEM). G indicates an overall genotype effect (p<0.05) and D indicates an overall dose effect (p<0.05). Post-hoc tests reveal significant genotype effects (*p<0.05), significant dose effects ($^{\#}$p<0.05) and doses significantly different from 0.00 mg/kg ($^{\$}$p<0.05).*

3.3 Acute anti-aggressive effect NLX-112 in WTG animals

In WTG animals, dose effects of NLX-112 were found in time spent on aggressive behavior ($F(5,132)=15.75$, $p<0.001$), attack latency ($F5,132)=21.85$, $p<0.001$) and time spent on social behavior ($F(5,132)=8.72$, $p<0.001$) (see Figure 3).

Relative to basal levels seen under vehicle treatment, aggression decreased at doses 0.16 ($t(23)=5.39$, $p<0.001$), 0.63 ($t(23)=6.48$, $p<0.001$) and 2.5 ($t(23)=5.65$, $p<0.001$) mg/kg. Consecutive dose differences in aggressive behavior were found at 0.04-0.16 ($t(23)=4.68$, $p<0.001$) and 0.16-0.63

(t(23)=2.19, p=0.039) mg/kg, where aggression decreased.

Relative to basal levels seen under vehicle treatment, attack latency increased at doses 0.16 (t(22)=-5.94, p<0.001), 0.63 (t(22)=-6.79, p<0.001) and 2.5 (t(22)=-6.64, p<0.001) mg/kg. Finally, an increase in attack latency (t(23)=-6.65, p<0.001) was found for 0.04-0.16 mg/kg.

Social behavior increased compared to basal levels seen under vehicle treatment at doses 0.16 (t(22)=-5.16, p<0.001), 0.63 (t(22)=-5.35, p<0.001) and 2.5 (t(22)=-3.76, p<0.001) mg/kg. Furthermore, an increase in social behavior was found at dose 0.04-0.16 mg/kg (t(23)=-5.02, p<0.001).

No time effects were found.

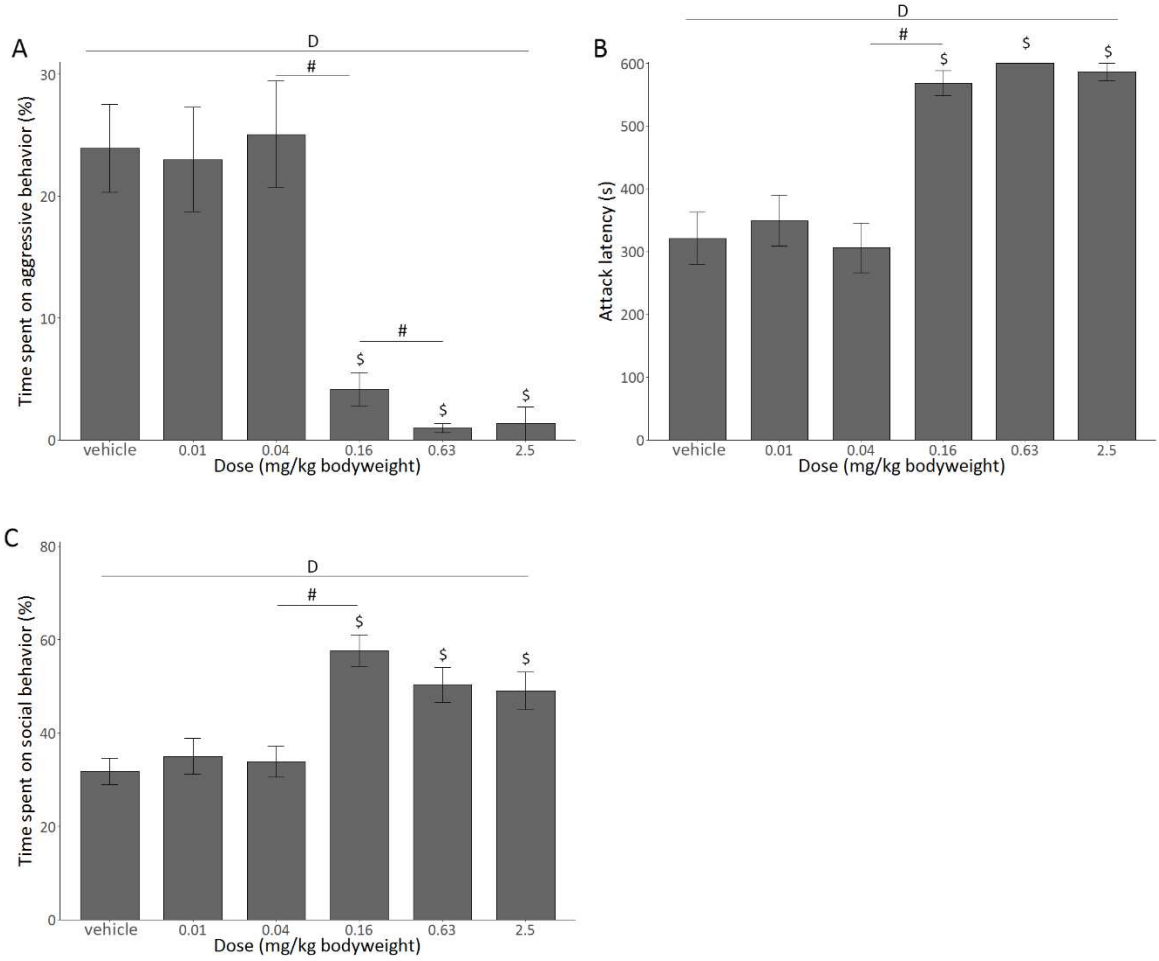


Figure 3: Effect of different dosages of NLX-112 (vehicle, 0.01, 0.04, 0.16, 0.63, 2.5 mg/kg bodyweight) on A) time spent on aggressive behavior, B) attack latency and C) time spent on social behavior in resident WTG rats (n=24) during a ten-minute interaction with an intruder animal. Data are represented as mean (±SEM). D indicates an overall dose effect (p<0.05). Post-hoc tests reveal significant dose effects ($^{\#}$p<0.05) and doses significantly different from 0.00 mg/kg ($^{\$}$p<0.05).

3.4 Dose-effect relationship

NLX-112 dose-effect curves were constructed by expressing aggressive behavior relative to the vehicle treatment (0 mg/kg bodyweight) to reliably compare the anti-aggressive potencies of NLX-112 between the different groups (e.g. Tph2 KO, Tph2 WT and WTG animals) (see Figure 4). A trend for a dose x group interaction was found ($F(8,190)=1.93$, $p=0.058$). Although this interaction was not significant, the dose-effect curve could be considered shifted rightward in the Tph2 KO as compared to the Tph2 WT and WTG. Furthermore, main effects for group ($F(2,190)=11$, $p<0.001$) and for dose ($F(4,190)=10.67$, $p<0.001$) were found. No time effect was found. Post hoc tests revealed an increased level of corrected aggressive behavior averaged over all NLX-112 doses in Tph2 KO animals when compared to Tph2 WT animals ($p=0.001$) and WTG animals ($p<0.001$). ED50 values for WTG, Tph2 WT and Tph2 KO animals were 0.13, 0.05 and 0.50 mg/kg respectively.

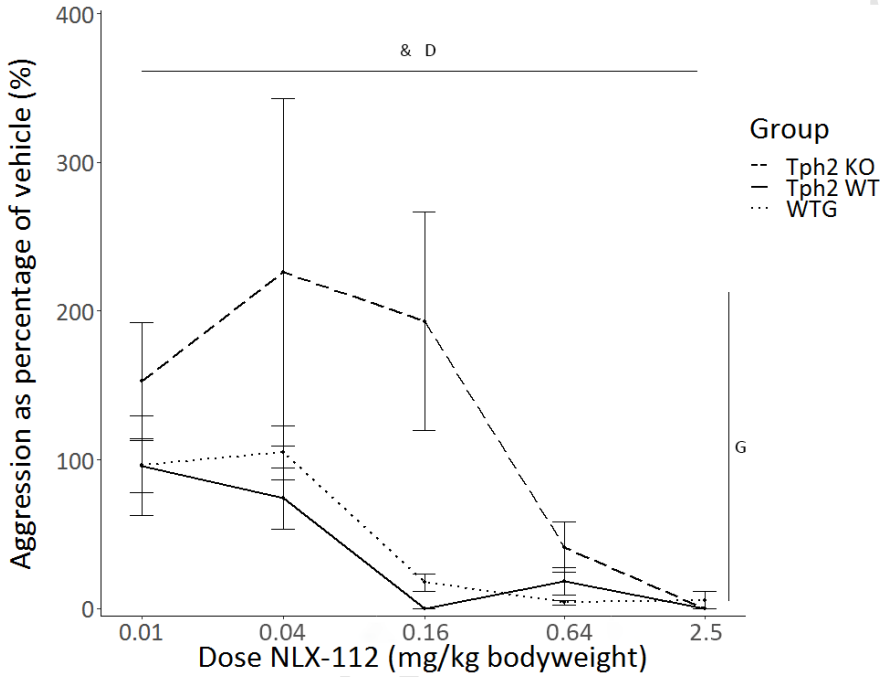


*Figure 4: Aggressive behavior per dosage of NLX-112 (0.01, 0.04, 0.16, 0.63, 2.5 mg/kg bodyweight) expressed as percentage of aggressive behavior relative to vehicle (0 mg/kg bodyweight). Data are represented as mean (±SEM), G indicates an overall group effect ($p<0.05$) and D indicates an overall dose effect ($p<0.05$) and & indicates a trend ($p<0.06$) in dose*group interaction.*

4. Discussion

The present study is the first, to our knowledge, to compare the aggressive behavior of Tph2KO and Tph2 WT rats. Several findings emerge from this study: firstly, the results indicate an enhanced aggressive phenotype in Tph2 KO, in comparison with Tph2 WT rats, as was anticipated based on the previously reported exaggerated aggressiveness in Tph2 KO mice (Mosienko et al., 2012). However, when compared to feral WTG rats, their aggression level is still significantly lower, and thus cannot be defined as exceptionally high or deviant. Secondly, a robust anti-aggressive effect of the highly

selective 5-HT_{1A} receptor agonist NLX-112 was found. Despite the even more pronounced difference in levels of aggressiveness between Tph2 WT and WTG rats, the dose-effect relationships appeared similar. Surprisingly however, and in contrast to what was expected, higher doses of NLX-112 were needed to reach the same anti-aggressive effect in the low serotonin Tph2 KO animals. This rightward shift in aggressive behavior in the dose-effect curve, suggests that 5-HT_{1A} receptor sensitivity is decreased in Tph2 KO rats compared to their WT counterparts and WTG rats.

This latter finding seems at odds with recent reports from literature where Tph2 KO mice demonstrate increased 5-HT_{1A} autoreceptor sensitivity. In these mice, lacking brain 5-HT synthesis, the mixed 5-$HT_{1A/7}$ receptor agonist 8-OHDPAT was shown to exert a higher suppression of serotonergic neuron firing, indicating increased 5-HT_{1A} autoreceptor sensitivity (Araragi et al., 2013; Mlinar et al., 2017). This increase could not be explained by upregulation of 5-HT_{1A} receptor expression, as data on this is shown to be inconsistent in Tph2 KO mice (Angoa-Pérez et al., 2012; Gutknecht et al., 2012; Jacobsen et al., 2012; Kim et al., 2014; Kriegebaum et al., 2010; Mosienko et al., 2014). However, a recent study in Tph2 KO mice showed that receptor expression is regulated independently of receptor functionality. Indeed, an increased functional coupling of 5-HT_{1A} autoreceptors to their principal effectors, G protein-coupled inwardly- rectifying potassium (GIRK) channels was observed, in the absence of an increase in 5-HT_{1A} receptor density (Mlinar et al., 2017).

Although serotonergic system formation seems to be normal in Tph2 KO mice (Gutknecht et al., 2012, 2008; Kriegebaum et al., 2010), the absence of brain 5-HT synthesis has been shown to decrease the number and density of GABAergic interneurons in the anterior basolateral amygdala (Waider et al., 2013). These interneurons are regulated by excitatory projections from the PFC, which are in turn regulated by the 5-HT_{1A} receptor (Jüngling et al., 2008; Quirk et al., 2003). Decreases in GABAergic interneurons may lead to a compensatory decrease in sensitivity of the presynaptic 5-HT_{1A} autoreceptors, thereby providing diminished serotonergic negative feedback inhibitory control. If such mechanisms are combined with a compensatory increase in postsynaptic 5-HT_{1A} receptor sensitivity, this would enable stabilization of serotonergic signaling in Tph2 KO animals. Obviously, further research is needed to resolve the seemingly opposite developmental adaptation mechanisms of 5-HT_{1A} autoreceptor sensitivity between rats and mice deficient in brain serotonin.

The Tph2 KO results in this study support an association between low 5-HT_{1A} receptor sensitivity and aggression. This is in line with findings in Norway rats bred for high affective aggressiveness, where a significant decrease in 5-HT_{1A} receptor binding in frontal cortex, hypothalamus and amygdala was found (Popova et al., 2005). In apparent clinical confirmation of these experimental findings, life-time aggression in a healthy human population of males and females correlated negatively with 5-HT_{1A}

binding *in vivo* in several brain regions (amongst others amygdala, dorsal raphe nucleus, orbital PFC) (Parsey et al., 2002). In contrast, another study in healthy human subjects showed a positive correlation between aggression and prefrontal 5-HT_{1A} binding potential as shown by PET scan (Witte et al., 2009). This is further supported by several studies in which enhanced postsynaptic receptor sensitivity was associated with high aggression in rodents (Korte et al., 1996; van der Vegt et al., 2001). These discrepancies might underlie the differences in 5-HT_{1A} receptor sensitivity and aggression shown here.

We showed an overall robust anti-aggressive effect of the 5-HT_{1A} receptor agonist NLX-112. This novel compound exhibits both high selectivity for the 5-HT_{1A} receptor as well as high agonist efficacy (Colpaert et al., 2002; Newman-Tancredi et al., 2017). As was shown here, these characteristics resulted in a specific anti-aggressive effect in Tph2 KO and WTG rats, while social behavior increased or stayed similar relative to the vehicle, indicating no major sedative or locomotor impairing effects of the drug treatment. A limitation of the present study is the relatively low aggressive behavior in Tph2 KO animals compared to baseline WTG aggression levels. This discrepancy could be explained by the increased anxiety found in the Dark Agouti background of Tph2 animals (Mechan et al., 2002), making the animals more vulnerable to stress from handling during injections. Furthermore, different routes of administration were used for the Tph2 (i.p.) and WTG (s.c.) experiments. Since both groups were habituated to the methods, no additional difference in stress of injection would be expected between the groups (Turner et al., 2011). Future studies should compare within one administration route and add a higher number of dosages, especially between 0.04-0.63 mg/kg, to provide a more exact dose-effect curve of aggressive behavior.

In conclusion, the robust anti-aggressive effect of the specific 5-HT_{1A} receptor agonist NLX-112 is demonstrated in Tph2 KO and WTG rats. This finding underlines the importance of the 5-HT_{1A} receptor in controlling aggressive behavior during an acute conflict situation, and demonstrates the potential to use the 5-HT_{1A} agonist NLX-112 (which is currently in clinical development) as a specific anti-aggressive treatment. The observation that high aggressive Tph2 KO rats show an increase in effective NLX-112 dose as compared to Tph2 WT and WTG animals, is indicative for a reduced 5-HT_{1A} autoreceptor sensitivity. It seems that receptor sensitivity in the absence of 5-HT plays an important role in aggressive behavior itself and its treatment. The current study suggests that 5-HT_{1A} receptor sensitivity in Tph2 KO rats is decreased compared to both Tph2 WT and WTG animals as opposed to previous reports in Tph2 KO mice, and that underlying mechanisms have to be further elucidated in future studies.

5. Conflicts of interest

A. Newman-Tancredi and M.A. Varney are employees and stock-holders of Neurolixis Inc. Other authors have no conflicts of interest to disclose.

6. Acknowledgement

This work was supported by a Junior Researcher grant of the Donders Institute, awarded to Judith R. Homberg and Robbert-Jan Verkes.

7. Supplementary materials

Supplementary table 1: Mean percentage of time spent on individual behavioral parameters (i.e. clinch attack, keep down, move towards aggressive, chase, lateral threat, upright aggressive, mounting, social behavior, move towards social, upright social, submissive behavior, no contact) in the ten-minute resident intruder interaction per dosage of NLX-112 (vehicle, 0.01, 0.04, 0.16, 0.63, 2.5 mg/kg bodyweight) in Tph2 KO (n=9), Tph2 WT (n=9) and WTG animals (n=24).

Tph2 KO

	Aggressive behavior								Social behavior				Other	
Dose	Clinch Attack	Keep Down	Move towards aggressive	Chase	Lateral threat	Upright aggressive	Mounting	Total aggressive behavior	Social behavior	Move towards social	Upright social	Total social behavior	Submissive behavior	No contact
vehicle	0,22	0,23	0,05	0,15	0,85	0,20	0,92	2,61	18,73	0,51	4,21	23,45	0,08	73,86
0,01	0,28	1,10	0,09	0,23	0,61	0,18	1,50	3,99	25,92	0,42	5,10	31,44	0,00	64,57
0,04	0,12	3,64	0,05	0,05	0,44	0,51	1,11	5,91	25,89	0,70	7,66	34,25	0,13	59,71
0,16	0,00	2,88	0,00	0,00	0,00	0,00	2,17	5,05	39,16	0,64	2,27	42,07	0,06	52,82
0,64	0,00	0,39	0,00	0,01	0,00	0,00	0,68	1,08	34,95	0,84	1,46	37,25	0,23	61,44
2,5	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	22,38	1,04	0,13	23,54	0,00	76,46

Tph2 WT

	Aggressive behavior								Social behavior				Other	
Dose	Clinch Attack	Keep Down	Move towards aggressive	Chase	Lateral threat	Upright aggressive	Mounting	Total aggressive behavior	Social behavior	Move towards social	Upright social	Total social behavior	Submissive behavior	No contact
vehicle	0,09	1,49	0,08	0,18	0,27	0,06	0,00	2,18	21,06	0,43	1,10	22,59	0,37	74,86
0,01	0,17	1,03	0,00	0,11	0,69	0,09	0,00	2,09	21,06	0,31	1,55	22,92	1,42	73,57
0,04	0,08	0,60	0,01	0,01	0,66	0,13	0,12	1,62	28,04	0,51	1,92	30,48	1,34	66,56
0,16	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	28,29	0,64	0,82	29,76	0,00	70,24
0,64	0,00	0,14	0,16	0,02	0,00	0,00	0,08	0,40	25,71	0,87	0,05	26,63	0,00	72,97
2,5	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	31,63	1,06	0,14	32,83	0,00	67,17

WTG

	Aggressive behavior								Social behavior				Other	
Dose	Clinch Attack	Keep Down	Move towards aggressive	Chase	Lateral threat	Upright aggressive	Mounting	Total aggressive behavior	Social behavior	Move towards social	Upright social	Total social behavior	Submissive behavior	No contact
vehicle	0,36	6,59	0,46	0,17	11,72	4,57	0,06	23,93	29,62	0,69	1,49	31,81	2,03	42,23
0,01	0,30	5,96	0,45	0,12	10,72	5,42	0,01	23,00	32,27	0,78	1,95	35,01	1,56	40,43
0,04	0,29	6,54	0,52	0,03	10,79	6,78	0,10	25,05	31,30	0,88	1,67	33,86	4,78	36,31
0,16	0,08	1,38	0,07	0,00	0,95	0,93	0,76	4,18	53,15	1,22	3,28	57,65	4,23	33,94
0,64	0,00	0,75	0,08	0,00	0,03	0,06	0,07	0,98	47,14	1,78	1,45	50,36	2,76	45,89
2,5	0,07	0,21	0,03	0,05	0,79	0,19	0,00	1,35	46,93	1,88	0,24	49,05	1,71	47,89

8. References

Angoa-Pérez, M., Kane, M.J., Briggs, D.I., Sykes, C.E., Shah, M.M., Francescutti, D.M., Rosenberg, D.R., Thomas, D.M., Kuhn, D.M., 2012. Genetic depletion of brain 5HT reveals a common molecular pathway mediating compulsivity and impulsivity. J. Neurochem. 121, 974–984. https://doi.org/10.1111/j.1471-4159.2012.07739.x

Araragi, N., Mlinar, B., Baccini, G., Gutknecht, L., Lesch, K.P., Corradetti, R., 2013. Conservation of 5-HT1Areceptor-mediated autoinhibition of serotonin (5-HT) neurons in mice with altered 5-HT homeostasis. Front. Pharmacol. 4 AUG, 1–11. https://doi.org/10.3389/fphar.2013.00097

Audero, E., Mlinar, B., Baccini, G., Skachokova, Z.K., Corradetti, R., Gross, C., 2013. Suppression of serotonin neuron firing increases aggression in mice. J. Neurosci. 33, 8678–88. https://doi.org/10.1523/JNEUROSCI.2067-12.2013

Beekman, M., Flachskamm, C., Linthorst, A.C.E., 2005. Effects of exposure to a predator on behaviour and serotonergic neurotransmission in different brain regions of C57bl/6N mice. Eur. J. Neurosci. 21, 2825–2836. https://doi.org/10.1111/j.1460-9568.2005.04107.x

Caldwell, E.E., Miczek, K.A., 2008. Long-term citalopram maintenance in mice: selective reduction of alcohol-heightened aggression. Psychopharmacology (Berl). 196, 407–16. https://doi.org/10.1007/s00213-007-0972-z

Caramaschi, D., de Boer, S.F., Koolhaas, J.M., 2007. Differential role of the 5-HT1Areceptor in aggressive and non-aggressive mice: An across-strain comparison. Physiol. Behav. 90, 590–601. https://doi.org/10.1016/j.physbeh.2006.11.010

Carrillo, M., Ricci, L.A., Coppersmith, G.A., Melloni, R.H., 2009. The effect of increased serotonergic neurotransmission on aggression: a critical meta-analytical review of preclinical studies. Psychopharmacology (Berl). 205, 349–368. https://doi.org/10.1007/s00213-009-1543-2

Celada, P., Puig, M. V, Casanovas, J.M., Guillazo, G., Artigas, F., 2001. Control of dorsal raphe serotonergic neurons by the medial prefrontal cortex: Involvement of serotonin-1A, GABA(A), and glutamate receptors. J Neurosci 21, 9917–9929. https://doi.org/21/24/9917 [pii]

Centenaro, L.A., Vieira, K., Zimmermann, N., Miczek, K.A., Lucion, A.B., De Almeida, R.M.M., 2008. Social instigation and aggressive behavior in mice: role of 5-HT1A and 5-HT1B receptors in the prefrontal cortex. Psychopharmacology (Berl). 201, 237–248. https://doi.org/10.1007/s00213-008-1269-6 [doi]

Coccaro, E.F., Steven, G., Siever, L.J., 1990. Buspirone challenge: Preliminary evidence for a role for central 5-HT1a receptor function in impulsive aggressive behavior in humans. Psychopharmacol. Bull. 26, 393–405.

Colpaert, F.C., Tarayre, J.P., Koek, W., Pauwels, P.J., Bardin, L., Xu, X.J., Wiesenfeld-Hallin, Z., Cosi, C., Carilla-Durand, E., Assié, M.B., Vacher, B., 2002. Large-amplitude 5-HT1Areceptor activation: A new mechanism of profound, central analgesia. Neuropharmacology 43, 945–958.

https://doi.org/10.1016/S0028-3908(02)00119-3

Comai, S., Tau, M., Pavlovic, Z., Gobbi, G., 2012. The Psychopharmacology of Aggressive Behavior : A Translational Approach Part 1: Neurobiology. J. Clin. Psychopharmacol. 32, 237–260. https://doi.org/10.1097/JCP.0b013e31824929d6

Cooper, M. a, Grober, M.S., Nicholas, C.R., Huhman, K.L., 2009. Aggressive encounters alter the activation of serotonergic neurons and the expression of 5-HT1A mRNA in the hamster dorsal raphe nucleus. Neuroscience 161, 680–90. https://doi.org/10.1016/j.neuroscience.2009.03.084

De Almeida, R.M.M., Lucion, A.B., 1997a. 8-OH-DPAT in the median raphe, dorsal periaqueductal gray and corticomedial amygdala nucleus decreases, but in the medial septal area it can increase maternal aggressive behavior in rats. Psychopharmacology (Berl). 134, 392–400. https://doi.org/10.1007/s002130050476

De Almeida, R.M.M., Lucion, A.B., 1997b. 8-OH-DPAT in the median raphe, dorsal periaqueductal gray and corticomedial amygdala nucleus decreases, but in the medial septal area it can increase maternal aggressive behavior in rats. Psychopharmacology (Berl). 134, 392–400. https://doi.org/10.1007/s002130050476

de Boer, S.F., Koolhaas, J.M., 2005. 5-HT1A and 5-HT1B receptor agonists and aggression: a pharmacological challenge of the serotonin deficiency hypothesis. Eur. J. Pharmacol. 526, 125–39. https://doi.org/10.1016/j.ejphar.2005.09.065

de Boer, S.F., Lesourd, M., Mocaër, E., Koolhaas, J.M., 2000. Somatodendritic 5-HT(1A) autoreceptors mediate the anti-aggressive actions of 5-HT(1A) receptor agonists in rats: an ethopharmacological study with S-15535, alnespirone, and WAY-100635. Neuropsychopharmacology 23, 20–33. https://doi.org/10.1016/S0893-133X(00)00092-0

De Boer, S.F., Newman-Tancredi, A., 2016. Anti-aggressive effects of the selective high-efficacy "biased" 5-HT1A receptor agonists F15599 and F13714 in male WTG rats. Psychopharmacology (Berl). 233, 937–947. https://doi.org/10.1007/s00213-015-4173-x

De Boer, S.F., Van der Vegt, B.J., Koolhaas, J.M., 2003. Individual variation in aggression of feral rodent strains: A standard for the genetics of aggression and violence? Behav. Genet. 33, 485–501. https://doi.org/10.1023/A:1025766415159

Duke, A. a, Bègue, L., Bell, R., Eisenlohr-Moul, T., 2013. Revisiting the serotonin-aggression relation in humans: a meta-analysis. Psychol. Bull. 139, 1148–72. https://doi.org/10.1037/a0031544

Finney, D., Tattersfield, F., 1952. Probit analysis.

Gutknecht, L., Araragi, N., Merker, S., Waider, J., Sommerlandt, F.M.J., Mlinar, B., Baccini, G., Mayer, U., Proft, F., Hamon, M., Schmitt, A.G., Corradetti, R., Lanfumey, L., Lesch, K.P., 2012. Impacts of brain serotonin deficiency following Tph2 inactivation on development and Raphe neuron serotonergic specification. PLoS One 7. https://doi.org/10.1371/journal.pone.0043157

Gutknecht, L., Waider, J., Kraft, S., Kriegebaum, C., Holtmann, B., Reif, A., Schmitt, A., Lesch, K.P., 2008. Deficiency of brain 5-HT synthesis but serotonergic neuron formation in Tph2 knockout mice. J. Neural Transm. 115, 1127–1132. https://doi.org/10.1007/s00702-008-0096-6

Hajo, Â., Sharp, T., 1999. Role of the medial prefrontal cortex in 5-HT 1A receptor-induced inhibition of 5-HT neuronal activity in the rat 1741–1750.

Holmes, A., Murphy, D.L., Crawley, J.N., 2002. Reduced aggression in mice lacking the serotonin transporter. Psychopharmacology (Berl). 161, 160–7. https://doi.org/10.1007/s00213-002-1024-3

Homberg, J.R., De Boer, S.F., Raasø, H.S., Olivier, J.D.A., Verheul, M., Ronken, E., Cools, A.R., Ellenbroek, B.A., Schoffelmeer, A.N.M., Vanderschuren, L.J.M.J., De Vries, T.J., Cuppen, E., 2008. Adaptations in pre- and postsynaptic 5-HT1A receptor function and cocaine supersensitivity in serotonin transporter knockout rats. Psychopharmacology (Berl). 200, 367–80. https://doi.org/10.1007/s00213-008-1212-x

Homberg, J.R., Pattij, T., Janssen, M.C.W., Ronken, E., De Boer, S.F., Schoffelmeer, A.N.M., Cuppen, E., 2007. Serotonin transporter deficiency in rats improves inhibitory control but not behavioural flexibility. Eur. J. Neurosci. 26, 2066–73. https://doi.org/10.1111/j.1460-9568.2007.05839.x

Jacobsen, J., Siesser, W., Sachs, B., Peterson, S., Cools, M., Setola, V., Folgering, J., Flik, G., Caron, M., 2012. Deficient serotonin neurotransmission and depression-like serotonin biomarker alterations in tryptophan hydroxylase 2 (Tph2) loss-of-function mice. Mol. Psychiatry 1, 233–245. https://doi.org/10.1016/j.dcn.2011.01.002.The

Jüngling, K., Seidenbecher, T., Sosulina, L., Lesting, J., Sangha, S., Clark, S.D., Okamura, N., Duangdao, D.M., Xu, Y.L., Reinscheid, R.K., Pape, H.C., 2008. Neuropeptide S-Mediated Control of Fear Expression and Extinction: Role of Intercalated GABAergic Neurons in the Amygdala. Neuron 59, 298–310. https://doi.org/10.1016/j.neuron.2008.07.002

Kaplan, K., Echert, A.E., Massat, B., Puissant, M.M., Palygin, O., Geurts, A.M., Hodges, M.R., 2016. Chronic central serotonin depletion attenuates ventilation and body temperature in young but

not adult Tph2 knockout rats. J. Appl. Physiol. jap.01015.2015. https://doi.org/10.1152/japplphysiol.01015.2015

Kim, J.Y., Kim, A., Zhao, Z.Q., Liu, X.Y., Chen, Z.F., 2014. Postnatal maintenance of the 5-Ht1a-Pet1 autoregulatory loop by serotonin in the raphe nuclei of the brainstem. Mol. Brain 7, 1–11. https://doi.org/10.1186/1756-6606-7-48

Koolhaas, J.M., Coppens, C.M., de Boer, S.F., Buwalda, B., Meerlo, P., Timmermans, P.J. a, 2013. The resident-intruder paradigm: a standardized test for aggression, violence and social stress. J. Vis. Exp. e4367. https://doi.org/10.3791/4367

Korte, S.M., Meijer, O.C., De Kloet, E.R., Buwalda, B., Keijser, J., Sluyter, F., Van Oortmerssen, G., Bohus, B., 1996. Enhanced 5-HT(1A) receptor expression in forebrain regions of aggressive house mice. Brain Res. 736, 338–343. https://doi.org/10.1016/0006-8993(96)00723-8

Kriegebaum, C., Song, N.-N., Gutknecht, L., Huang, Y., Schmitt, A., Reif, A., Ding, Y.-Q., Lesch, K.-P., 2010. Brain-specific conditional and time-specific inducible Tph2 knockout mice possess normal serotonergic gene expression in the absence of serotonin during adult life. Neurochem. Int. https://doi.org/10.1016/j.neuint.2010.06.015

Mechan, A.O., Moran, P.M., Elliott, M.J., Young, A.M., Joseph, M.H., Green, R.A., 2002. A comparison between Dark Agouti and Sprague-Dawley rats in their behaviour on the elevated plus-maze, open-field apparatus and activity meters, and their response to diazepam. Psychopharmacology (Berl). 159, 188–195. https://doi.org/10.1007/s002130100902

Miczek, K.A., Hussain, S., Faccidomo, S., 1998. Alcohol-heightened aggression in mice: Attenuation by 5-HT(1A) receptor agonists. Psychopharmacology (Berl). 139, 160–168. https://doi.org/10.1007/s002130050701

Mlinar, B., Montalbano, A., Waider, J., Lesch, K.P., Corradetti, R., 2017. Increased functional coupling of 5-HT1A autoreceptors to GIRK channels in Tph2-/- mice. Eur. Neuropsychopharmacol. 27, 1258–1267. https://doi.org/10.1016/j.euroneuro.2017.10.033

Montoya, E.R., Terburg, D., Bos, P.A., van Honk, J., 2012. Testosterone, cortisol, and serotonin as key regulators of social aggression: A review and theoretical perspective. Motiv. Emot. 36, 65–73. https://doi.org/10.1007/s11031-011-9264-3

Mos, J., Olivier, B., Poth, M., Van Oorschot, R., Van Aken, H., 1993. The effects of dorsal raphe administration of eltoprazine, TFMPP and 8-OH-DPAT on resident intruder aggression in the rat. Eur. J. Pharmacol. 238, 411–415. https://doi.org/10.1016/0014-2999(93)90877-K

Mosienko, V., Bert, B., Beis, D., Matthes, S., Fink, H., Bader, M., Alenina, N., 2012. Exaggerated aggression and decreased anxiety in mice deficient in brain serotonin. Transl. Psychiatry 2, e122. https://doi.org/10.1038/tp.2012.44

Mosienko, V., Matthes, S., Hirth, N., Beis, D., Flinders, M., Bader, M., Hansson, A.C., Alenina, N., 2014. Adaptive changes in serotonin metabolism preserve normal behavior in mice with reduced TPH2 activity. Neuropharmacology 85, 73–80. https://doi.org/10.1016/j.neuropharm.2014.05.015

Newman-Tancredi, A., Depoortère, R., Carilla-Durand, E., Tarayre, J.P., Kleven, M., Koek, W., Bardin, L., Varney, M.A., 2017. NLX-112, a highly selective 5-HT1Areceptor agonist: Effects on body temperature and plasma corticosterone levels in rats. Pharmacol. Biochem. Behav. 165, 56–62. https://doi.org/10.1016/j.pbb.2017.11.002

Nichols, David E., C.D.N., 2008. Serotonin Receptors. Chem. rewiews 108(5) 1614–41 1614–1641.

Olivier, B., 2004. Serotonin and aggression. Ann. N. Y. Acad. Sci. 1036, 382–92. https://doi.org/10.1196/annals.1330.022

Parsey, R. V, Oquendo, M.A., Simpson, N.R., Ogden, R.T., Heertum, R. Van, Arango, V., Mann, J.J., 2002. E ffects of sex, age, and aggressive traits in man on brain serotonin 5-HT receptor binding potential measured by PET using 1A [C-11]WAY-100635. Brain Res. 954, 173–182. https://doi.org/10.1016/S0006-8993(02)03243-2

Perreault, H.A.N., Semsar, K., Godwin, J., 2003. Fluoxetine treatment decreases territorial aggression in a coral reef fish. Physiol. Behav. 79, 719–724. https://doi.org/10.1016/S0031-9384(03)00211-7

Pineyro, G., Blier, P., 1999. Autoregulation of serotonin neurons: role in antidepressant drug action. Pharmacol. Rev. 51, 533–591.

Pinna, G., Dong, E., Matsumoto, K., Costa, E., Guidotti, A., 2003. In socially isolated mice, the reversal of brain allopregnanolone down-regulation mediates the anti-aggressive action of fluoxetine. Proc. Natl. Acad. Sci. U. S. A. 100, 2035–40. https://doi.org/10.1073/pnas.0337642100

Popova, N.K., Naumenko, V.S., Plyusnina, I.Z., Kulikov, A. V., 2005. Reduction in 5-HT1A receptor density, 5-HT1A mRNA expression, and functional correlates for 5-HT1A receptors in genetically defined aggressive rats. J. Neurosci. Res. 80, 286–292. https://doi.org/10.1002/jnr.20456

Quirk, G.J., Likhtik, E., Pelletier, J.G., Paré, D., 2003. Stimulation of medial prefrontal cortex decreases the responsiveness of central amygdala output neurons. J. Neurosci. 23, 8800–7.

https://doi.org/23/25/8800 [pii]

Rosell, D.R., Siever, L.J., 2015. The neurobiology of aggression and violence. CNS Spectr. 20, 254–279. https://doi.org/10.1017/S109285291500019X

Sakaue, M., Ago, Y., Sowa, C., Sakamoto, Y., Nishihara, B., Koyama, Y., Baba, A., Matsuda, T., 2002. Modulation by 5-hT2A receptors of aggressive behavior in isolated mice. Jpn. J. Pharmacol. 89, 89–92.

Sánchez, C., Meier, E., 1997. Behavioral profiles of SSRIs in animal models of depression, anxiety and aggression. Are they all alike? Psychopharmacology (Berl). 129, 197–205. https://doi.org/10.1007/s002130050181

Schiller, L., Donix, M., Jähkel, M., Oehler, J., 2006. Serotonin 1A and 2A receptor densities, neurochemical and behavioural characteristics in two closely related mice strains after long-term isolation. Prog. Neuro-Psychopharmacology Biol. Psychiatry 30, 492–503. https://doi.org/10.1016/j.pnpbp.2005.11.008

Snoeren, E.M.S., 2010. In search for animal models of female sexual dysfunction.

Stein, D.J., Miczek, K.A., Lucion, A.B., De Almeida, R.M.M., 2013. Aggression-reducing effects of F15599, a novel selective 5-HT1A receptor agonist, after microinjection into the ventral orbital prefrontal cortex, but not in infralimbic cortex in male mice. Psychopharmacology (Berl). 230, 375–387. https://doi.org/10.1007/s00213-013-3164-z

Takahashi, A., Quadros, I.M., Almeida, R.M.M., Miczek, K.A., 2011a. Behavioral and Pharmacogenetics of Aggressive Behavior. Psychopharmacology (Berl). 213, 73–138. https://doi.org/10.1007/7854

Takahashi, A., Quadros, I.M., Almeida, R.M.M., Miczek, K.A., 2011b. Behavioral and Pharmacogenetics of Aggressive Behavior. Psychopharmacology (Berl). 213, 73–138. https://doi.org/10.1007/7854

Turner, P. V, Brabb, T., Pekow, C., Vasbinder, M.A., 2011. Administration of substances to laboratory animals: routes of administration and factors to consider. J. Am. Assoc. Lab. Anim. Sci. 50, 600–13.

van der Vegt, B.J., de Boer, S.F., Buwalda, B., de Ruiter, a J., de Jong, J.G., Koolhaas, J.M., 2001. Enhanced sensitivity of postsynaptic serotonin-1A receptors in rats and mice with high trait aggression. Physiol. Behav. 74, 205–11.

van der Vegt, B.J., Lieuwes, N., van de Wall, E.H.E.M., Kato, K., Moya-Albiol, L., Martínez-Sanchis, S., de Boer, S.F., Koolhaas, J.M., 2003. Activation of serotonergic neurotransmission during the performance of aggressive behavior in rats. Behav. Neurosci. 117, 667–674. https://doi.org/10.1037/0735-7044.117.4.667

van Erp, a M., Miczek, K. a, 2000. Aggressive behavior, increased accumbal dopamine, and decreased cortical serotonin in rats. J. Neurosci. 20, 9320–5.

Waider, J., Proft, F., Langlhofer, G., Asan, E., Lesch, K.P., Gutknecht, L., 2013. GABA concentration and GABAergic neuron populations in limbic areas are differentially altered by brain serotonin deficiency in Tph2 knockout mice. Histochem. Cell Biol. 139, 267–281. https://doi.org/10.1007/s00418-012-1029-x

Witte, a V., Flöel, A., Stein, P., Savli, M., Mien, L.-K., Wadsak, W., Spindelegger, C., Moser, U., Fink, M., Hahn, A., Mitterhauser, M., Kletter, K., Kasper, S., Lanzenberger, R., 2009. Aggression is related to frontal serotonin-1A receptor distribution as revealed by PET in healthy subjects. Hum. Brain Mapp. 30, 2558–70. https://doi.org/10.1002/hbm.20687

Highlights

- Tph2 KO rats show increased aggressive behaviour compared to their WT counterparts
- The 5-HT_{1A} receptor agonist NLX-112 shows a robust anti-aggressive effect
- The dose-effect curve shifts rightward in Tph2 KO as compared to WT and WTG rats
- This suggests a decreased 5-HT_{1A} receptor sensitivity in Tph2 KO rats

Conflicts of interest

A. Newman-Tancredi and M.A. Varney are employees and stock-holders of Neurolixis Inc. Other authors have no conflicts of interest to disclose.